Cell Host & Microbe
Meeting Review

Microbial Pathogenesis: Mechanisms of Infectious Disease

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The FASEB Summer Research Conference on Microbial Pathogenesis: Mechanisms of Infectious Disease was held in Colorado, USA in July 2007. The central theme was the interplay between pathogenic microbes and their mammalian hosts. Here, we review the presented research that highlights this theme, including studies of both short-term and long-term interactions between microbes and their hosts.

Introduction

The mountain splendor of Snowmass, Colorado provided a perfect setting for the 2007 FASEB Summer Research Conference on Microbial Pathogenesis: Mechanisms of Infectious Disease. This meeting, chaired by Brad Cookson (University of Washington, USA) and cochaired by Michele Swanson (University of Michigan, USA), brought together a diverse group of immunologists, bacteriologists, virologists, parasitologists, and mycologists in an atmosphere conducive to open discussion and idea exchange. The central theme of the meeting was the interplay between pathogenic microbes and their mammalian hosts.

Appropriate for the theme, the Bernard Fields Keynote Lecture was given by Nobel Laureate Peter Doherty (University of Melbourne, Australia). Dr. Doherty enchanted the audience by recounting his years spent investigating the regulation of infection by T cells. He discussed his long-standing interest in the factors that influence the quality and quantity of $CD8^+$ T cell responses to infection and vaccination. His talk was peppered with entertaining personal tales about his experience in science, and he assured young researchers that anyone with a "fire in their belly" could become a Nobel Laureate.

The Initial Interaction between Microbes and Their Hosts

Just over a decade ago, the late Charles Janeway led a renewed interest in the importance of innate immunity as the first and critical line of defense against infectious agents. Janeway proposed that the innate immune system, although more limited in scope and unable to be fine-tuned for precise specificity like the adaptive immune system, recognizes specific (but common) pathogen-associated molecular patterns (PAMPs) using individual pathogen-recognition receptors (PRRs). The discovery of Toll as a PRR in *Drosophila* was followed quickly by the identification in mammals of extracellular Toll-like receptors (TLRs) and intracellular Nod-like receptors (NLRs) (see Sansonetti [2006] for a recent review on this topic). While engagement of these receptors leads to activation of NF-κB and the induction of proinflammatory cytokine gene expression, stimulation can also lead to cell death via the activation of caspase-1 and formation of "inflammasomes." Several talks at this meeting focused on understanding various aspects of innate response to infection and the interactions of pathogens with immune cells (Figure 1).

As part of its pathogenic strategy, the Gram-negative bacterium *Francisella tularensis* enters and grows in the cytosol of host cells, including macrophages (Henry and Monack, 2007). Denise Monack (Stanford University, USA) showed that *F. tularensis* activates an ASC adaptor-containing inflammasome in bone marrow-derived macrophages leading to pyroptosis (a cell death pathway that is proinflammatory and uniquely dependent on caspase-1). However, using a microarray approach, her group showed that a type I interferon (IFN) response was also induced by *Francisella* and that the IFN response was required for the activation of the inflammasome. These results suggest a potentially global role for IFN-β as a positive activator of the inflammasome, revealing a previously unappreciated connection between these host response pathways. Another Gram-negative bacterium, *Legionella pneumophila*, grows within a specialized vacuole of macrophages that forms in response to proteins secreted by *Legionella*'s Type 4 Secretion System (T4SS) (Nagai and Roy, 2003). Craig Roy (Yale University, USA) showed that macrophages use TLR-2 to sense extracellular *L. pneumophila*, resulting in the induction of IFN-γ, and Nod1 and Nod2 to detect intracellular bacterial components, leading to the production of proinflammatory cytokines. Using MyD88- and RIP2-deficient mice and T4SS-deficient bacteria, his group also showed that additional receptors must be involved in sensing *L. pneumophila*, and together, their data suggest that, by integrating signals received through multiple receptors, host cells are able to evaluate the location, composition, and pathogenic strategy of invading microbes. That host cells integrate signals from multiple pathogen-sensing receptors was also evident from work

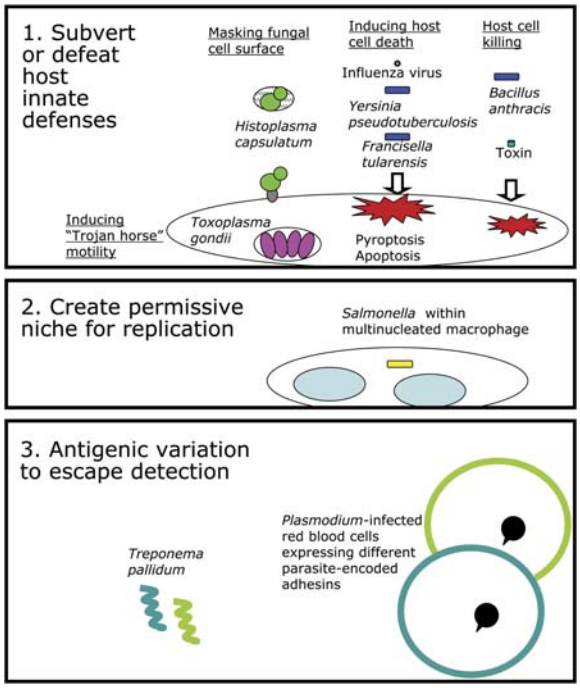

Figure 1. Themes in Pathogen Interactions with Immune Cells

on Dectin-1 signaling that was presented by David Underhill (Cedars Sinai/UCLA, USA). Dectin-1 is a non-TLR receptor that recognizes β-glucan, a component of the cell wall of the yeast form of *Candida albicans* and the mycelial form of *Aspergillus fumigatus* (Dennehy and Brown, 2007). Engagement of Dectin-1 stimulates phagocytosis of the fungi. Dr. Underhill showed that in addition to enhancing signaling through TLR-2, Dectin-1 induces signaling through the Src/Syk and Nuclear Factor of Activated T cells (NFAT) pathways, which are generally associated with adaptive immune responses. As one example of how microbes have learned to avoid innate host defenses, William Goldman (Washington University-St. Louis, USA) showed that the dimorphic fungal pathogen *Histoplasma capsulatum* covers the β-glucan on its surface with α-(1,3)-glucan when it switches to the pathogenic yeast form, thereby avoiding detection by Dectin-1 and subsequent phagocytosis.

Yersinia spp. are Gram-negative bacteria that induce apoptosis in naive macrophages in a manner dependent on TLR-4 and YopJ, an effector secreted by *Yersinia*'s Type 3 Secretion System (T3SS) (Bliska, 2006). Tessa Bergsbaken (University of Washington, USA) showed, however, that when macrophages were activated with LPS or lipopeptide, *Y. pseudotuberculosis* induced pyroptosis instead of apoptosis. Induction of pyroptosis required a functional T3SS but none of the known T3SS-dependent effectors, suggesting that either an unidentified effector or T3SS-dependent membrane perturbations were responsible for activating the inflammasome. These data suggest that *Yersinia* may induce apoptosis early in infection and then pyroptosis later, after activated macrophages have been recruited to the site of infection. This hypothesis was supported by data presented by Peter Dube (University of Texas Health Sciences Center, USA) and Wyndham Lathem (Washington University-St. Louis, USA) who have independently been studying the early phases of pneumonic plague using *Y. pestis* and a mouse model. Dr. Dube showed that inflammation was suppressed during the first 24–36 hr of infection, coincident with the induction of IL-10, but then increased rapidly as IL-10 was replaced with proinflammatory cytokines MIP-2 and keratinocyte chemoattractant (KC) in lung tissues. Dr. Lathem showed that increased expression of plasminogen activator protease (Pla) by *Y. pestis* correlated with the transition from the quiescent phase to the proinflammatory phase, although its role in the transition is currently unknown. While the molecular mechanisms are not yet understood, these data together indicate that both the pathogen and the host make important contributions to controlling the initial inflammatory response and the subsequent course of the infection.

Whether viruses induce pyroptosis is unknown, but viral-induced cell death is apparent. Stacey Schultz-Cherry (University of Wisconsin, USA) discussed the role of cell death in influenza pathogenesis. Two cellular pathways identified as involved in influenza virus-induced apoptosis were regulated during influenza infection. Downregulating both cellular pathways led to more severe disease and enhanced inflammation, suggesting that host cell apoptosis may be an important means of regulating the inflammatory response.

Jayne Raper (New York University, USA) described a completely different innate immune defense mechanism that kills trypanosomes growing in the bloodstream. A subfraction of high-density lipoprotein in the blood of humans and a few primates, called trypanosome lytic factor (TLF), is endocytosed by certain trypanosomes and is activated within the acidic lysosome, forming membrane pores that kill the parasite (Raper et al., 2001). Dr. Raper's group generated transgenic mice expressing human TLF and showed that, in addition to being less sensitive to the trypanosomatid *Leishmania major*, these mice were also less sensitive to *Salmonella typhimurium*, a Gram-negative bacterium that survives within macrophage phagosomes. These results suggest that TLF is a broad-spectrum antimicrobial component of the human innate immune system.

Secretory immunoglobulin (sIg) is a polyspecific, low-affinity antibody that protects mucosal surfaces (Wijburg et al., 2006). The polymeric immunoglobulin receptor (pIgR) is responsible for the transit of sIg through the epithelium. Richard Strugnell (University of Melbourne, Australia) reported that mice lacking pIgR are more susceptible to low doses of orally inoculated *Salmonella typhimurium* and more readily spread the organism to other mice. Disturbances in the intestinal tract of the pIgR mouse suggest that sIg participates in removal of antigen from the subepithelial spaces.

The use of inbred "wild-type" and transgenic mice raised in pathogen-free environments has greatly

facilitated research, especially that aimed at understanding microbe-host interactions. However, in nature, hosts are neither genetically homogeneous nor free of current or past exposure to other pathogens. Janis Weis (University of Utah, USA) showed that C3H mice develop joint inflammation near the site of intradermal inoculation with *Borrelia burgdorferi*, the agent of Lyme disease in humans, and that infected tissue revealed strong upregulation of interferon response genes (IRG). In contrast, C57BL/6 mice, which develop only mild disease, showed decreased expression of IRG and instead activated an epidermal differentiation/wound repair response. Although their inflammatory responses and the severity of disease differed, both strains of mice activated an effective bacterial clearance response involving NF-κB signaling. These results serve as an excellent reminder that data obtained using one strain of mouse may not translate directly to others, much less to other mammals, and therefore extrapolating conclusions to human infection should be done with caution.

Manuela Raffatellu (University of California-Davis, USA) showed that the ability of *S. typhimurium* to disseminate to mesenteric lymph nodes following inoculation of ligated ileal loops was greatly increased in SIV-infected Rhesus macaques compared to uninfected animals. SIV-infected animals displayed a blunted cytokine response to *Salmonella*, consistent with SIV-dependent depletion of gut T_h17 cells. These observations demonstrate how infection by one pathogen can alter the host immune response to infection with another.

While it is apparent that many microbes participate in an intimate two-way communication with host cells, some simply deliver potent toxins capable of quickly killing host cells. Erik Hewlett (University of Virginia, USA) presented evidence that the adenylate cyclase toxins (ACT) produced by *Bordetella pertussis* and *Bacillus anthracis* kill macrophages by both apoptotic and nonapoptotic pathways. Although the exact mechanisms are not yet understood, cAMP accumulation, ATP depletion, and pore formation all appear to be involved. Using a yeast model, Dara Frank (Medical College of Wisconsin, USA) showed that ExoU, an effector secreted by the *Pseudomonas aeruginosa* T3SS, functions as an A_2 phospholipase in eukaryotic cells inducing necrotic cell death. Intriguingly, ExoU only had phospholipase activity in the presence of a host cell factor, which was shown to be superoxide dismutase (SOD1). ExoU and SOD1 interact, and the dismutase activity of SOD1 was not required for ExoU phospholipase activity. Thus, not only does *P. aeruginosa* kill host cells, it uses a host cell protein as a cofactor to do so. Using *C. elegans* as a model, Daniel Kalman (Emory University, USA) discovered that enteropathogenic *Escherichia coli* (EPEC) produces a small molecule that paralyzes and ultimately kills the nematodes. In addition, worms exposed briefly to EPEC, were ''conditioned'' (or ''immunized'') and survived a subsequent lethal exposure. This conditioning required expression of dopaminergic neurotransmitters and the FOX-O family transcription factor, DAF-16, which regulates expression of genes encoding protective factors. These signaling pathways are highly conserved across species and their role in mammalian systems is being pursued.

Secondary Interactions: Adaptive Immunity Kicks In

The adaptive immune system is critical for controlling pathogens that are able to overcome innate responses and for eliminating these same pathogens quickly upon subsequent exposure. The mounting of a secondary response that is faster and more robust than the first requires the development of memory T cells. John Harty (University of Iowa, USA), by studying the biology of $CD8^+$ T cell-mediated immunity to the bacterium *Listeria monocytogenes*, has demonstrated that it takes ~40 days for a significant population of memory T cells to develop. This time could be shortened, however, if dendritic cells coated with *Listeria* peptides were administered at the time of primary infection or vaccination. These elegant studies demonstrated that the rate at which $CD8^+$ T cells acquire memory depends on the degree of inflammation and can be manipulated to improve the efficacy of prime and boost vaccination.

Given the importance of T cell-mediated control of viral infection, it is surprising how little is known about the modulation of T cell function by viruses, especially herpesviruses. Ann Hill (Oregon Health & Science University, USA) demonstrated that cells infected with cytomegaloviruses (CMV) are destroyed by CD8+ T cells if they express a high enough level of MHC-I, but that a long interaction time is required. Noncognate MHC-I-peptide complexes played an important role in facilitating lysis. Not surprisingly, CMV encode multiple genes that inhibit the ability of cytotoxic T lymphocytes to lyse infected targets. Andrew Evans (Emory University, USA) demonstrated that a murine gammaherpesvirus encodes a secreted protein that stimulates T cells expressing Vβ4+ receptors specifically, reminiscent of the effects of other viral or bacterial superantigens. This unique T cell response appears to limit viral reactivation among specific cell types via IFN-γ, thus promoting viral latency in a manner independent of the antigen-specific immune response. Such T cell modulation is not without negative consequences, however, as this viral protein appears to mediate immunopathology in certain knockout strains of mice.

Hepatitis C virus (HCV) is persistent and prevalent, affecting over 3% of the world population, and notoriously difficult to study. Using lymphocytes from blood and liver of HCV-infected patients, Kyong-Mi Chang (University of Pennsylvania and Philadelphia VA, USA) examined regulatory and effector T cells in HCV-infected patients. Her work provided evidence that virus-specific T cells, but not B cells, are associated with viral clearance during acute infection. Further, numerous immune regulatory mechanisms were apparently activated in HCV-infected patients and a hierarchy in antiviral $CD8^+$ T cells was observed.

$CD8^+$ T cell responses are generally not associated with extracellular pathogens but exciting work by Molly Bergman (Tufts University, USA) demonstrated that $CD8^+$ T

cells are required for protection from *Y. pseudotuberculosis*. Moreover, the host protein perforin, which is involved in cytotoxic T cell-mediated lysis of infected cells, was also required to limit bacterial growth. These results suggest that immune mechanisms that typically target intracellular pathogens also function to eliminate extracellular pathogens.

The ability of microbes to change their surface properties to avoid recognition and clearance by the adaptive immune response, called antigenic variation, has been known for many years, and in some cases the underlying genetic mechanisms are understood (van der Woude and Baumler, 2004). Investigating the mechanism of antigenic variation in *Treponema pallidum*, the causal agent of syphilis, has been challenging, but Sheila Lukehart (University of Washington, USA) has discovered that a *T. pallidum* surface protein called TprK, a target of opsonic antibodies, undergoes phase variation in response to immune pressure, and that the underlying mechanism involves segmental gene conversion from adjacent chromosomal variable region cassettes. Another form of phase variation occurs when erythrocytes infected with the malaria parasite *Plasmodium* express one of ~60 polymorphic cytoadherence genes encoding PfEMP1 on the infected cell surface. Switching between *PfEMP1* genes precludes antibody neutralization. Kirk Dietsch (Cornell University, USA) reported that exclusive expression of one *PfEMP1* gene from its upstream promoter is dictated by a silencing mechanism involving intron-dependent expression of a noncoding, "sterile" RNA from each *PfEMP1* gene. Both silencing and recognition for mutually exclusive expression depend on the upstream promoter and intron promoter being in a one-to-one ratio, suggesting that interactions between the two are required for proper epigenetic regulation. Although flagellar phase variation in *Salmonella* species is generally well understood, Stephen Baker (Wellcome Trust Sanger Institute, UK) described two novel flagella variants that exist in *Salmonella* strains isolated from Indonesia. In one type, genes resembling the regulators of phase variation in other *Salmonella* subspecies are located on a linear plasmid. Differences in flagella type correlated with differences in invasiveness of the organisms for cultured cells.

Long-Term Interactions: Commensalism, Chronic Infection, Latency

In some instances, the responses of the host to the presence of an invader are balanced by the strategies used by the organism to resist clearance, and the result is a standoff in which the microbe persists within the host in the absence of overt disease. This microbial colonization may represent a commensal relationship in which the organism is considered a component of the normal flora. An example is colonization of the avian intestinal tract by the enteric bacterium *Campylobacter jejuni.* As described by Victor DiRita (University of Michigan, USA), colonization requires *C. jejuni* genes involved in flagellar motility, nutrient acquisition, and surprisingly, protein N-glycosylation (Hendrixson and DiRita, 2004). Although uncommon in most bacteria, over 30 *C. jejuni* proteins are N-glycosylated. A putative zinc-binding, N-glycosylated periplasmic protein that may be involved in zinc acquisition is needed for normal colonization. Some of the glycoproteins are also required for invasion of a human intestinal epithelial cell line, suggesting that determinants of colonization in the chicken also promote disease when the organism is ingested by an "accidental" host such as a human.

The opportunistic fungal pathogen *Candida albicans* has a complex relationship with its human host, colonizing as a commensal but capable of becoming an invasive pathogen if the host becomes immunocompromised. C.A.K. (Tufts University, USA) described a gene encoding a putative transcription factor that is expressed by fungal cells colonizing the intestinal tract of mice in the absence of disease. Genetic manipulation of this gene resulted in changes in intestinal colonization, but the gene was not required for lethality following intravenous inoculation of *C. albicans*. The converse situation, genes required for normal lethality in the intravenous model but not for intestinal colonization, was also described. These results suggest that, for *C. albicans*, the commensal state is distinct from the pathogenic state, and each has a characteristic genetic program.

Other pathogens utilize specialized mechanisms that allow them to persist for long periods of time within the host. *Mycobacterium tuberculosis*, for example, can remain dormant within macrophages, with reactivation resulting in disease many years after the primary exposure. Clifford Harding (Case Western Reserve University, USA) presented evidence that *M. tuberculosis* actively inhibits expression of class II MHC on macrophages (Pecora et al., 2006) by decreasing the expression of CIITA, a transcriptional activator required for sustained expression of MHC-II. This strategy promotes persistence in these cells while avoiding detection by $CD4^+$ T cells. Correlia Detweiler (University of Colorado, USA) described a mechanism used for long-term persistence by *Salmonella typhimurium*, an organism associated with persistent infection of carriers. In a murine host, persisting organisms were detected within macrophages that contained other cell types, including neutrophils and T cells. In laboratory experiments, bacteria were shown to survive and replicate within activated bone marrow-derived macrophages that had ingested other live, but not dead, cells such as T cells. Intriguingly, "hemophagocytosis" (defined as the engulfment of erythrocytes, leukocytes, platelets, and precursor cells by activated macrophages) has been described in humans suffering from typhoid fever and may aid the survival of *Salmonella*.

An extreme example of a standoff is viral latency. In this situation, the virus exists in a latent state for long periods of time, potentially for the life span of the host, and remains capable of reactivating to produce active disease. Although latent infection represents a quiescent stage in the viral life cycle, Michael Lagunoff (University of Washington, USA) reported that latent infection with Kaposi's sarcoma-associated herpesvirus (KSHV) converts blood

endothelial cells into lymphatic endothelial cells (Carroll et al., 2004) in part by upregulating the lymphangiogenic marker Vascular Endothelial Growth Factor Receptor 3 (VEGF R3) via activation of STAT3 signaling. In addition, activation of the hypoxia transcription factors HIF1α and HIF2α triggers expression of the angiogenic receptor VEGF R1. Activation of these angiogenic and lymphangiogenic pathways may contribute to the prominent vascularization of Kaposi's sarcomas.

Special Topics

Environmental Sensing: How Do the Pathogens Know Where They Are?

Pathogens must be proficient at sensing their surroundings and responding to cues that promote survival in the face of a changing environment. Dimorphic ("two-phase") pathogenic fungi (e.g., *Blastomyces dermatitidis* and *Histoplasma capsulatum*) sense an increase in temperature to undergo a dramatic phase transition from a mycelial form in soil to a yeast form in the human lung. Bruce Klein (University of Wisconsin, USA) described how this transition is critically dependent on a phosphorelay-type signaling system in which the hybrid sensor Drk1 (dimorphism regulating kinase 1) phosphorylates a response regulator (similar to Ssk1 in *S. cerevisiae*), ultimately driving expression of yeast-specific virulence factors such as the *B. dermatitidis* adhesin Bad1 (Nemecek et al., 2006).

Rachel Edwards (University of Michigan, USA) described how *Legionella pneumophila* uses a ppGpp synthase called SpoT to monitor fatty acid (FA) metabolism. Perturbation of FA metabolism cues replicative cells to differentiate to a growth-arrested, motile, infectious form equipped for transmission from one host cell to another. Inhibitors that selectively block initiation or elongation of FA trigger phase transition in a SpoT-dependent manner, as do short-chain FA such as acetic acid or proprionic acid. Although it seems likely that SpoT senses perturbations in FA biosynthesis through its known interaction with acyl carrier protein, precisely how it does this and which genes respond to elevation of ppGpp remain active areas of investigation.

Enterohemorrhagic *E. coli* (EHEC) also uses a sophisticated surveillance system to taste cues from its host and the surrounding gut microflora. Vanessa Sperandio (University of Texas-Southwestern, USA) showed that EHEC senses increasing host epinephrine (ep) and norepinephrine (norep) levels to activate virulence gene expression. It also monitors levels of autoinducer-3 (AI-3) produced by itself and other gut microflora to express virulence traits such as attachment and effacement. Although ep/norep and AI-3 appear to recognize the same bacterial receptor, downstream signaling occurs through distinct multicomponent phosphotransfer systems regulating the expression kinetics of these traits (Reading et al., 2007; Russell et al., 2007; Sharp and Sperandio, 2007). It is proposed that signaling to initiate flagellum-based movement to the gut epithelium occurs before signaling that activates attachment and effacement. A small-molecule antagonist (LED209) of AI-3 signaling identified in a high-throughput screen blocks EHEC effacement and also inhibits macrophage infection with *Francisella tularensis*, raising the exciting prospect of selectively downregulating virulence as a treatment strategy.

Manipulating Host Cell Functions to Get In and Move Around

Intracellular pathogens are adept at subverting host cell processes to their advantage. *Brucella abortus* is an intracellular bacterium that replicates within a vacuole whose membrane is derived from the endoplasmic reticulum. Jean Celli (NIAID, USA) showed that, prior to residing within a mature ER-derived vacuole that is capable of supporting *Brucella* replication, the organism resides in an intermediate vacuole that acquires lysosomal markers and intersects with the endocytic system. Maturation of the intermediate vacuole to the mature vacuole requires acidification and is Rab7 dependent.

Isabelle Coppens (Johns Hopkins University, USA) showed that the intracellular protozoan *Toxoplasma gondii* becomes encased in a network of host microtubules and lysosomes, some of which can be seen encroaching within the parasitophorous vacuole (PV) but still surrounded by the PV membrane (Coppens et al., 2006). It is proposed that cholesterol and other nutrients are transferred from the encroaching lysosomes into the PV where they can be consumed by the parasite. In this manner the parasite selectively obtains essential nutrients without risking exposure to hydrolytic enzymes of the host. *Toxoplasma* is highly adept at crossing biological barriers such as the blood brain barrier or the placenta during dissemination and transmission. To understand the mechanism that allows the organisms to breach these barriers, Dr. Antonio Barragan (Karolinska Institute, Sweden) analyzed the motility of the parasite and of infected host cells. Results showed that intracellular parasites stimulated hypermotility in dendritic cells (DC) and other cells (Lambert et al., 2006). In mice inoculated with *Toxoplasma*-ladened DC, faster dissemination and more severe infection were observed in comparison to mice inoculated with the organism alone. These results suggest that *Toxoplasma* uses DC and other migratory immune cells as a "Trojan horse" to slip through biological barriers to access privileged tissues and organs.

Discovery of New Virulence Factors

Like many parasites, *Plasmodium*, the agent of malaria, has a complex life cycle. Consequently, a large fraction of *Plasmodium* genes are of unknown function. To gain insight into their functions, Elizabeth Winzeler (Scripps Institute, USA) described approaches that utilize gene expression data generated from organisms grown under a wide variety of conditions. By observing coexpression of genes of unknown function with genes of known function, Dr. Winzeler was able to generate and test hypotheses for the function of many unknown genes.

Inspection of the genome sequence of the flea-transmitted bacterium *Yersinia pestis* revealed the presence of a cluster of genes with homology to insecticidal toxin-encoding genes from *Photorhabdus luminescens*. Carleen Collins (University of Washington, USA) showed that the

encoded proteins are produced at 26°C but not 37°C, they are deployed by a T3SS, and together they have tyrosine phosphatase activity. Flea infection experiments indicate that these proteins may contribute to blockage of the flea proventriculus, thereby facilitating transmission by the flea vector.

Autotransporters (AT) are proteins that appear to mediate their own export across the outer membranes of Gram-negative bacteria (Henderson et al., 2004). Nearly all that have been studied play roles in pathogenesis. The *Y. pestis* genome contains 12 uncharacterized ORFs with the potential to encode ATs. Matthew Lawrenz (Washington University, USA) described evidence that these genes (collectively called *yaps*) are expressed during mammalian infection by *Y. pestis*, and that several localize to the outer membrane when expressed in *E. coli*. By constructing and characterizing *Y. pestis* strains with in-frame deletion mutations in these genes, he showed that at least four play a role in the infection and that one, *yapE*, is important for dissemination of *Y. pestis* from the lymph nodes to deeper tissues such as the spleen and lungs.

New Vesicular Trafficking Pathways and Tools

David Haslam (Washington University, USA) described using a luciferase reporter system in a high-throughput screen to identify small molecules that inhibit the transport of microbial toxins, such as Shiga toxin, inside eukaryotic cells. The screen identified a compound, called B06, that interferes with the budding of vesicles from the Golgi and TGN. B06 appears to do this by inhibiting the polymerization of Arf1-coat complexes into an oligomeric lattice. Because B06 affects a different step in vesicular transport than Brefeldin A, it will be a useful tool for investigating intracellular transport pathways, which are often exploited by pathogenic microbes.

New Vaccination Strategies

Vaccination strategies often target healthy populations and standard vaccines may not work in special populations such as neonates, the elderly, pregnant women, or immunocompromised individuals. Marcela Pasetti (University of Maryland, USA) presented a new vaccination strategy that exploits the properties of particles derived from killed *Lactococcus lactis* organisms. These particles elicit responses in dendritic cells from neonatal mice or from human umbilical cord blood offering potential as a means to develop vaccines for infants and neonates.

Conclusions

The host-pathogen interaction is an intricate "dance." Sometimes the pathogen leads, and other times it is the host that is in control. Ultimately, the interactions lead to a resolution favoring one or the other or to a balanced situation in which the two coexist. This meeting emphasized the importance of the interactions and the ability of both parties to respond to each other. What was most apparent is that pathogens, regardless of the type, use similar mechanisms to evade and control the host immune response. The discussions at this meeting promoted the appreciation that pathogens may differ in many ways, but they are also similar. Through sharing of knowledge and collaborations between investigators with diverse expertise but a general focus on pathogenesis, we may one day uncover the keys to preventing infectious diseases, protecting susceptible individuals, or improving therapeutic interventions.

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